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Methods for the Synthesis of L-Leucine Selectively Labelled with Carbon-13 or Deuterium in either Diastereotopic Methyl Group

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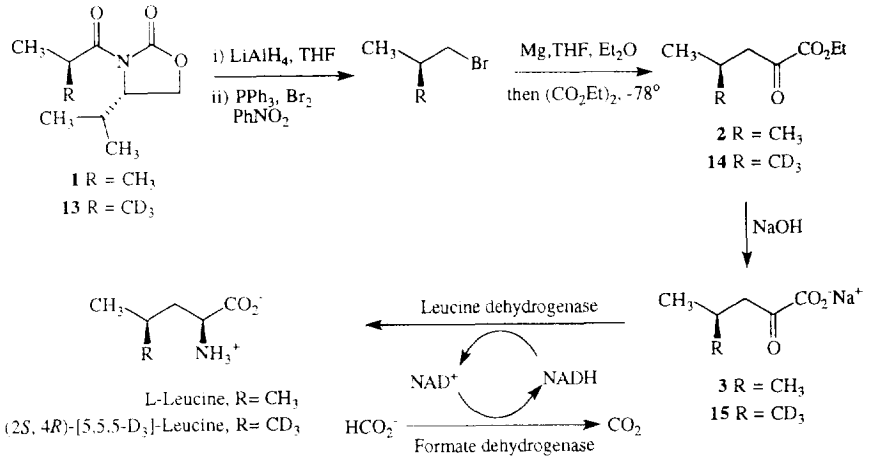
Abstract : A versatile approach is described for the enantioselective synthesis of isotopically labelled L-leucine involving the preparation of 2-oxo-4-methylpentanoic acid labelled selectively with carbon-13 or deuterium in either the *pro-R* or *pro-S* methyl group followed by a reductive amination of the ketone catalysed by leucine dehydrogenase. This strategy is applied to the total synthesis of (2*S*,4*R*)-[5,5,5-D_3]-leucine using CD_3I as the source of deuterium.

The availability of isotopically labelled amino acids in enantiomerically pure form is valuable in a range of studies in bioorganic chemistry and in particular to investigate the 3-dimensional structure of proteins by NMR spectroscopy. The incorporation of amino acids selectively labelled with carbon-13, deuterium and nitrogen-15 into the protein combined with heteronuclear NMR experiments facilitate the assignments of the complex ^{1}H-NMR resonances of proteins. In the light of the importance of these probes, several methods for the synthesis of L-leucine specifically labelled in either the *pro-R* or *pro-S* methyl groups have been reported including the preparation of (2*S*,4*R*)-[5,5,5-D_3]-leucine from either L-pyroglutamic acid[1] or (*R*)-pulegone[2] and the synthesis of (4*R*)- and (4*S*)-[5-^{13}C]-leucine using a combination of chemical and enzymatic resolution techniques[3,4]. We now report a more general approach for the efficient synthesis of L-leucine which has the advantage that it enables the stereoselective labelling of either diastereotopic methyl group with carbon-13 or deuterium as well as the incorporation of carbon-13 at C-3 and C-4.

Results and Discussion

We have recently described a simple method for the efficient synthesis of [^{15}N]-L-leucine via the reductive amination of α-keto-isocaproate catalysed by leucine dehydrogenase[5]. By analogy, a flexible approach to the synthesis of carbon-13 and deuterated L-leucine would be via incubation of the appropriately labelled α-keto acid with leucine dehydrogenase. Many methods have been reported for the preparation of α–keto acids[6] and of particular value is reaction of diethyl oxalate with a Grignard reagent at low temperature.

Therefore in order to use this approach for the synthesis of isotopically labelled L-leucine, we required 2-methyl-1-bromopropane specifically labelled with carbon-13 or deuterium in either diastereotopic methyl group and we favoured the use of Evans chiral auxiliaries[7] to achieve this goal. The conversion of **1** into L-leucine was first carried out on unlabelled material to establish the viability of the proposed approach (Scheme 1).



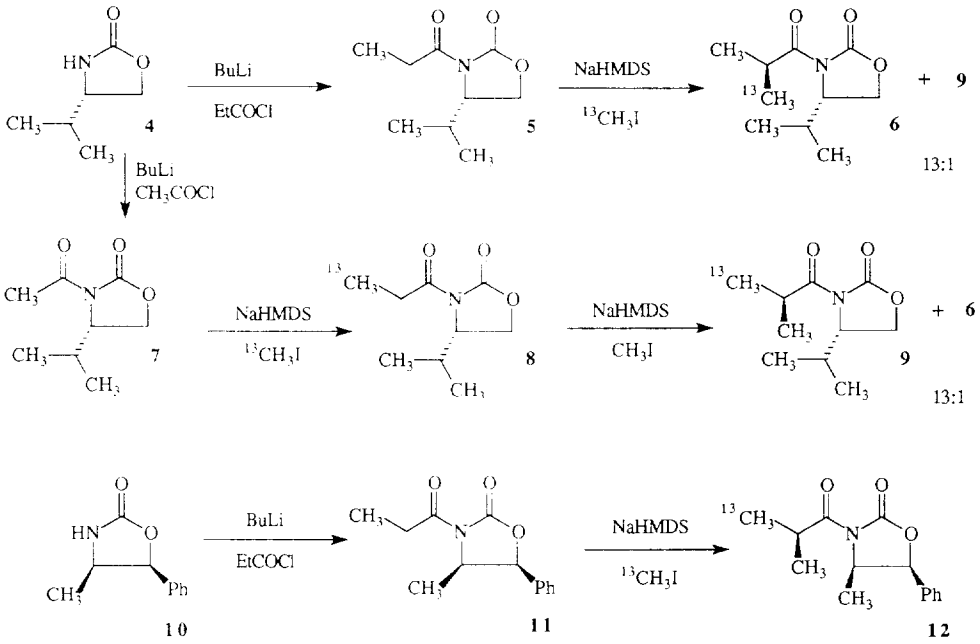
Scheme 1

Reductive cleavage of the chiral auxiliary in **1** with lithium aluminium hydride followed by treatment of the resultant alcohol with triphenylphosphine and bromine gave the required 2-methyl-1-bromopropane as the sole product in 72% yield over the two steps (Scheme 1)[8]. Treatment of the bromide with magnesium in THF gave the Grignard reagent which was reacted *in situ* with diethyl oxalate at -78°C to give the α-keto ester **2** in good yield. Saponification of the ethyl ester with sodium hydroxide followed by reductive amination of the α-keto acid **3** catalysed by leucine dehydrogenase using the protocol described previously[5] gave L-leucine in 85% yield from **2**[9].

Having established the viability of the synthetic route on unlabelled material, the next stage of our investigations involved an examination of the diastereoselectivity of the alkylation of (4*S*)-3-propionyl-4-isopropyloxazolidinone **5** using isotopically labelled methyl iodide. Although the alkylation of **5** with a wide range of electrophiles has been previously described[7], to the best of our knowledge the reaction with isotopically labelled methyl iodide has not been reported. The diastereoselectivity was established using ^{1}H-NMR spectroscopy on the carbon-13 analogues as follows (Scheme 2).

Reaction of (4*S*)-4-isopropyloxazolidinone **4** with *n*-butyl lithium followed by propionyl chloride gave the propionyl derivative **5** in 91% yield. The optimum conditions for the alkylation of **5** with methyl iodide proved to be generation of the enolate with sodium hexamethyldisilazide and quenching with 10 equivalents

of methyl iodide giving **1** in 90% yield. However to minimise the quantity of expensive isotopically labelled CH_3I, further optimisation showed that 1.4 equivalents of methyl iodide giving a 76% yield of **6** was the best compromise. The alternative diastereomer, **9**, was prepared from (4*S*)-3-acetyl-4-isopropyloxazolidinone **7** by an initial alkylation with $^{13}CH_3I$ to give the [^{13}C]-propionyl derivative **8** followed by alkylation with CH_3I as shown in Scheme 2.

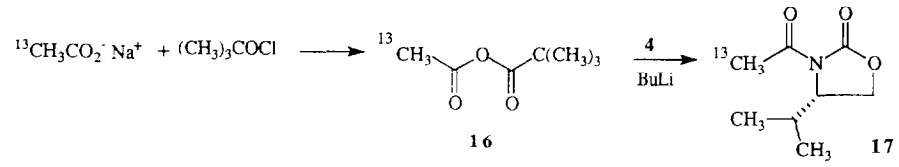


Scheme 2

In the ^{1}H-NMR spectra of **6** and **9**, the signals due to 2'-CH_3 and 2'-$^{13}CH_3$ were well resolved due to the large ^{13}C-^{1}H coupling, and integration of the signals at δ 1.22 (dd, J 128.3, 6.9Hz in **6**; dd, J 6.9, 5.0Hz in **9**) and at δ 1.15 (dd, J 6.7, 5.2 in **6**; dd, J 127.9, 6.7Hz in **9**) confirmed that selective alkylation of (4*S*)-3-propionyl-4-isopropyloxazolidinone had occurred giving a 13:1 mixture of diastereomers. A more direct route to the precursor of (2*S*,4*R*)-[5-^{13}C]-leucine involved the use of the (+)-norephedrine derived chiral auxiliary[7], giving **12** as a 13:1 mixture of diastereomers (Scheme 2).

Having established a general approach for the preparation of isotopically labelled L-leucine, the method was applied to the total synthesis of (2*S*, 4*R*)-[5,5,5-D_3]-leucine. Alkylation of **5** with CD_3I gave **13** in 72 % yield (Scheme 1). Reductive cleavage of the chiral auxiliary followed by conversion of the resultant alcohol to the α-keto ester **14** proceeded smoothly. Saponification of the ester to followed by reductive amination of the resultant α-keto acid **15** catalysed by leucine dehydrogenase gave (2*S*,4*R*)-[5,5,5-D_3]-leucine. The ^{1}H-NMR spectrum of L-leucine at 500MHz showed doublets (J 7Hz) at δ 0.87 and δ 0.89 assigned to the *pro-R* and *pro-S* methyl groups respectively. In the ^{1}H-NMR spectrum of (2*S*,4*R*)-[5,5,5-D_3]-leucine only the doublet at δ 0.87 was apparent.

This chemo-enzymatic approach to the synthesis of L-leucine has the further advantage that it enables the incorporation of carbon-13 at C-3 and/ or C-4 using [^{13}C]sodium acetate as the source of isotopic label. For example, treatment of [2-^{13}C]-sodium acetate with pivaloyl chloride gives the mixed anhydride **16**. Reaction of **16** with the lithium salt of **4** gives the acylated product **17** in 70% yield (Scheme 3), the precursor of [4-^{13}C]-leucine. In addition, the approach may also be modified for the inclusion of nitrogen-15[5].



Scheme 3

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8. General method for preparation of 2-methyl-1-brompropane: Br_2 (0.31ml, 6mmol) was added to Ph_3P (1.6g, 6mmol) in nitrobenzene (30ml) under N_2 and stirred for 10 minutes at room temp. The alcohol (0.46ml, 5mmol) in nitrobenzene (5 ml) was added and the mixture heated to 70°C for 2h. Quinoline (0.71ml, 6mmol) was added to the mixture at room temp. and stirred for 15 minutes and then the bromide was distilled on a manifold to a trap at -196°C. The bromide was redistilled through a P_2O_5 bulb and THF (6ml) added and used immediately in the formation of the Grignard reagent.
9. All compounds gave the expected NMR and mass specral data and optical rotations were in good agreement with literature values of unlabelled material.

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